263-Pos Board B43
Modulation of the α-Crystallin Chaperon Activity Induced by Changes in the Exposed Surface
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Small heat shock proteins (sHSPs) are highly conserved proteins, capable of chaperone activity under stress conditions, that is, able to bind unfolded substrates in response to pH, temperature, or other stress stimuli and prevent their aggregation and precipitation [1-2].
The α-crystallin family of sHSPs is involved in several neurological, muscular, and ophthalmic pathologies. This family includes the vertebrate lens protein α-crystallin, associated with cataract disease. In this study, by combining small angle X-ray [4] and light scattering techniques [5], the structure and shape of α-crystallin was revealed in its native state and after a transition caused by heat stress. Below critical temperature (Tc), α-crystallin appears as an ellipsoid with a central cavity; whereas at high temperatures the cavity almost disappears, and the protein rearranges its structure, increasing the solvent exposed surface while retaining the ellipsoidal symmetry. Contextually, at Tc, α-crystallin chaperone binding shows an abrupt increase. By modelling the chaperone activity as the formation of a complex composed of α-crystallin and an aggregating substrate, it was demonstrated that the increase of α-crystallin exposed surface is directly responsible for its gain in chaperone functionality.
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264-Pos Board B44
Chaperones Rescue Luciferase Folding by Separating its Domains
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Over the last 50 years significant progress has been made toward understanding how small single-domain proteins fold, however very little is known about folding mechanisms of medium and large multidomain proteins that predominate the proteomes of all forms of life. Large proteins frequently fold cotranslationally and/or require chaperones. Firefly (Photinus pyralis) luciferase (Luciferase) (550 residues) has been a model of a cotranslationally folding protein whose extremely slow refolding (~days) is catalyzed by chaperones. However, the mechanism by which Luciferase misfolds and how chaperones assist Luciferase refolding remains unknown. Here we combine single-molecule force-spectroscopy (AFM-SMFS) with computer simulations (SMD) to unravel the mechanism of chaperone-assisted Luciferase refolding. Our AFM and SMD results show that partially unfolded Luciferase - with the N-terminal domain remaining folded - can robustly refold without chaperones. Complete unfolding causes Luciferase to get trapped in very stable non-native configurations involving interactions between N- and C-terminal residues. However, chaperones allow the completely unfolded Luciferase to refold quickly in AFM experiments, strongly suggesting that chaperones are able to sequester non-natively contacting residues. More generally, we suggest that many chaperones, rather than actively promoting the folding, mimic the ribosomal exit tunnel and physically separate protein domains allowing them to fold in a cotranslational-like sequential process.

265-Pos Board B45
Inter-Domain Dynamics of a Novel Chaperone Enables Effective Capture of Membrane Protein Substrates
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Protein homeostasis is essential for all cells and requires proper control of the folding, localization and interactions of proteins. The biogenesis of membrane proteins poses a particular challenge to the protein homeostasis network, wherein molecular chaperones play an important role by preventing or reversing membrane protein aggregation, maintaining them in translocation competent states before they reach the membrane destination.
The light harvesting chlorophyll binding proteins (LHCPs) comprise the most abundant family of membrane proteins on earth. LHCPs are made in cytosol and, en route to the thylakoid membrane, they need to be imported into the chloroplast stroma and captured by the chloroplast signal recognition particle (cpSRP), comprised of cpSRP43 and cpSRP54. It has been shown that cpSRP43 is an effective molecular chaperone that not only prevents but also effectively reverses the aggregation of its substrate, LHCP, using ATP-independent binding interactions.
cpSRP43 provides the first proof-of-principle example to demonstrate that efficient reversal of protein aggregation can be attained by adequate binding between a chaperone and its substrate protein. However, little is known about how this chaperone binds and protects the transmembrane (TM) domain of its substrate and how the inter-domain dynamics activates its chaperone activity. Using solution NMR, we mapped the TM binding sites of cpSRP43. We further provided evidence for an inter-domain conformational change in this chaperone that activates it for effective capture of the membrane protein substrate. This inter-domain dynamics may provide a mechanism for timed capture and release of membrane protein during their localization.

Protein-Small Molecule Interactions I

266-Pos Board B46
Determination of Biomolecular Interactions using Microscale Thermophoresis
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The analysis of biomolecular interactions is important in the identification of therapeutic targets and development of diagnostics, as well as providing insights into cellular processes. MicroScale Thermophoresis (MST), an immobilization-free technology, is used to quantitate biomolecular interactions (pM-mM), ranging from protein-protein interactions to small molecule-target binding. MST, the directed movement of molecules in optically generated microscopic temperature gradients, is monitored by fluorescence. This thermophoretic movement is affected by the entropy of the hydration shell around molecules and is highly sensitive to binding reactions, which affect the size, charge, conformation, and/or hydration shell. We show how MST can be used to identify and quantify interactions between biomolecules of interest: proteins, nucleic acids, ions, etc. We also demonstrate how interactions with proteins can be analyzed in a Label-Free manner using tryptophan fluorescence. With MST, one can also probe affinities in close-to-native conditions: in detergent, liposomes, cell lysate, or blood serum.

267-Pos Board B47
Rational Design of Surface Modified Nanoparticles for Modulation of Amyloid Beta Aggregation
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Alzheimer's disease (AD) is a debilitating disease that is the sixth leading cause of death in the United States. It is the only disease that is in the top ten most lethal that currently has no known cure. One of the hallmarks of AD is the presence of amyloid-β (Aβ) aggregates in the brains of those afflicted. Current understanding of the etiology of AD points to general disruption of the aggregation pathway as a promising strategy for a potential cure.
Surface modified nanoparticles have demonstrated striking effectiveness as inhibitors of Aβ aggregation. We have observed inhibition of Aβ aggregation by polyacrylic acid-coated gold nanospheres at substoichiometric ratios as low as 1:2,000,000. Nanoparticles coated with weak polyelectrolytes can produce significant local effects on pH of which Aβ aggregation is highly dependent. It is through this mechanism that we propose that these nanospheres can inhibit with such potency. A molecular theory has been developed in which intramolecular interactions are treated exactly and intermolecular interactions are treated within a mean-field approximation. The theory also accounts for the effects of curvature in the system present on the molecular scale with very good agreement with experimental data.
When tethered to a surface, weak polyelectrolytes confine electric charge. To mitigate the resulting energetically unfavorable electrostatic repulsions, the polymer layer of polyacrylic-acid coated nanospheres will recruit hydronium ions in weakly ionic aqueous solutions, locally lowering the pH. When the same conditions are used to parameterize the theoretical model as were used in experiment, the resulting sphere of lowered pH is pervasive enough to cause the observed abrogation of Aβ aggregation. Together, surface modified nanoparticles with this robust and accurate molecular model represent a powerful platform to engineer nanotechologies to modulate Aβ aggregation.